USEPA REGION 9 LABORATORY
RICHMOND, CALIFORNIA

STANDARD OPERATING PROCEDURE 275
EXTRACTION OF WATER SAMPLES BY CONTINUOUS LIQUID-LIQUID EXTRACTION

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1 SCOPE AND APPLICABILITY

This standard operating procedure (SOP) describes the extraction of semivolatile organic compounds (SVOCs), organochlorine pesticides, polychlorinated biphenyls (PCBs), and hydrocarbon fuels or oils from aqueous samples by continuous liquid-liquid extraction. This SOP is based on procedures in EPA SW-846 Method 3520C, Continuous Liquid-Liquid Extraction, Revision 3, December 1996 and is applicable to finished water, groundwater, and wastewater. Deviations from the reference method are listed in Appendix A.

These extracts may be analyzed using the following USEPA Region 9 Laboratory SOPs: 315 *Semivolatile Organics Analysis*, 330 *Organochlorine Pesticides by Gas Chromatography*, 335 *Polychlorinated Biphenyls (PCBs) as Aroclors by GC/ECD*, and 385 *Extractable Petroleum Hydrocarbons by GC/FID*. The applicability of these procedures to specific project data quality objectives (DQOs) must be assessed on a case-by-case basis.

2 METHOD SUMMARY

A measured volume of aqueous sample, approximately one liter, is placed in a continuous liquid-liquid extractor. The sample is pH-adjusted as necessary, fortified with a surrogate solution, and extracted with dichloromethane for 18 to 24 hours.

The extracts are dried with sodium sulfate. Extracts for extractable petroleum hydrocarbons are concentrated to 3.0 mL using a Kuderna-Danish apparatus; extracts for SVOC, including 1,4-dioxane are concentrated to 1 mL using a Kuderna-Danish apparatus and nitrogen evaporation.

Extracts for organochlorine pesticides and PCBs analysis are dried with sodium sulfate, solvent exchanged for hexane, and then concentrated to 10.0 mL using a Kuderna-Danish apparatus and nitrogen evaporation. Extracts may be cleaned up using Florisil cartridges as necessary. Extracts prepared for the analysis of PCBs may undergo acid cleanup prior to analysis.

3 DEFINITIONS

Analytical Sample – any sample in which analytes are being determined, excluding standards, blanks, or QC reference samples.

Demonstration of Capability (DOC) – A demonstration that the laboratory can produce data of acceptable quality. A DOC consists of three elements:

- An approved SOP (for which each analyst is trained),
- A method detection limit study completed by the analyst, and

- A precision and accuracy study completed by the analyst.

Extraction Batch – A group of up to twenty samples or less extracted together and sharing the same batch QC samples.

Laboratory Control Sample (LCS) – An aliquot of blank matrix to which known quantities of the method analytes are added. The LCS is analyzed like a sample, and its purpose is to determine whether the methodology is in control, and whether the Laboratory is capable of making accurate and precise measurements. The LCS is also known as a laboratory fortified blank (LFB) or blank spike (BS).

LIMS – Laboratory Information Management System. The Element database.

Matrix Spike (MS) / Matrix Spike Duplicate (MSD) – aliquots of an analytical sample to which known quantities of the method analytes are added. The MS/MSD is analyzed exactly like a sample; its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS/MSD corrected for background concentrations.

Method Blank (MB) – An aliquot of blank matrix that is treated exactly as a sample. The MB is used to detect sample contamination resulting from the procedures used to prepare and analyze the samples in the laboratory environment. The MB is also known as laboratory reagent blank (LRB).

PCBs – Polychlorinated Biphenyls, reported as Aroclors.

PEST – organochlorine pesticides

Surrogate – Compounds which are extremely unlikely to be found in any sample that are added to a sample aliquot in a known amount before extraction or other processing, and measured with the same procedures used to measure other sample components. The purpose of the surrogate is to monitor method performance with each sample.

Sample Delivery Group (SDG) – Typically, a group of twenty samples or less from a project that is sent to the laboratory for analysis. The number of samples in an SDG may exceed 20 samples depending on laboratory or project needs.

SVOC – Semi-Volatile Organic Compounds. Certain extractable compounds included in the SW-846 Method 8270 list of analytes and including 1,4-dioxane.

TPH Extractable (TPH/E) – Total Petroleum Hydrocarbons, also referred to as extractable petroleum hydrocarbons. Diesel and motor oil range hydrocarbons.

4 SAFETY & HEALTH

All laboratory personnel must follow health and safety requirements outlined in current versions of the EPA Region 9 Laboratory Chemical Hygiene Plan and the Region 9 Laboratory Business Plan. Potential hazards specific to this SOP as well as pollution prevention and waste management requirements are described in the following sections.

4.1 Chemical Hazards

Due to the unknown and potentially hazardous characteristics of samples, all sample handling and preparation should be performed in a well-vented laboratory fume hood.

The toxicity and carcinogenicity of each reagent used in this method may not be fully established. Each chemical should be regarded as a potential health hazard and exposure to them should be minimized by good laboratory practices. Refer to the Material Safety Data Sheets located in Room 118 (library) and the LAN for additional information.

Safety precautions must be taken when handling solutions and samples. Protective clothing including lab coats, safety glasses, and gloves must always be worn. Contact lenses must not be worn. If solutions come into contact with your eyes, flush with water continuously for 15 minutes. If solutions come in contact with your skin, wash thoroughly with soap and water. ESAT personnel should contact the Group Leader or Health and Safety and Environmental Compliance Task Manager and EPA staff should see the Team Leader or the Laboratory Safety, Health and Environmental Compliance Manager to determine if additional treatment is required. Refer to the Material Safety Data Sheets located in the library and the LAN for additional information.

Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens. Stock standard solutions of these compounds must be prepared in a fume hood. Routine procedures in this SOP do not require contact with concentrated solutions or neat materials.

4.1.1 Dichloromethane

Dichloromethane is a suspected carcinogen. Effects of overexposure: acute inhalation or ingestion causes mild central nervous system depression. The primary toxic effect is narcosis. Other toxic effects are pulmonary edema, encephalopathy, and hemolysis. Dichloromethane irritates the eyes, skin, and respiratory tract. No systemic effects have been reported in humans, although excessive concentrations have caused cancer as well as liver and kidney damage in animals.

Emergency and first aid – Inhalation: immediately remove to fresh air. If not breathing, administer mouth-to-mouth rescue breathing. If there is no pulse,

administer cardiopulmonary resuscitation (CPR), contact physician immediately. Eye contact: rinse with copious amounts of water for at least 15 minutes. Get emergency medical assistance. Skin contact: flush thoroughly for at least 15 minutes. Wash affected skin with soap and water. Remove contaminated clothing and shoes. Wash clothing before re-use, and discard contaminated shoes. Get emergency medical assistance. Ingestion: call local poison control center for assistance. Contact physician immediately. Never induce vomiting or give anything by mouth to a victim unconscious or having convulsions.

4.1.2 Hexane

Hexane liquid and vapors are extremely flammable; keep it away from ignition sources. Hexane is harmful if inhaled or swallowed and may cause damage to kidneys, nerves, and respiratory system. It is irritating to skin, eyes, mucous membranes, and is toxic if ingested inhaled. Vapor inhalation causes irritation of nasal and respiratory passages, headache, dizziness, nausea, and central nervous system depression. Chronic overexposure can cause severe nerve damage. No systemic toxicity has been reported.

Emergency First Aid – Inhalation: immediately remove to fresh air. If not breathing, administer mouth-to-mouth rescue breathing. If there is no pulse, administer CPR. Contact physician immediately. Eye contact: Rinse with copious amounts of water for at least 15 minutes. Get emergency medical assistance. Skin contact: Flush thoroughly for at least 15 minutes. Wash affected skin with soap and water. Remove contaminated clothing and shoes. Wash clothing before re-use, and discard contaminated shoes. Get emergency medical assistance. Ingestion: Call local Poison Control Center for assistance. Contact physician immediately. Aspiration hazard: do not induce vomiting.

4.1.3 Sulfuric Acid

Sulfuric acid is a corrosive poison. Liquid and mist cause severe burns to all body tissues and may be fatal if swallowed or inhaled. Inhalation produces damaging effects on the mucous membranes and upper respiratory tract. Symptoms of exposure by inhalation may include irritation of the nose and throat, and labored breathing. Do not get acid in eyes, on skin, or on clothing. Skin contact can cause redness, pain, and severe skin burns. In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes.

When diluting an acid, the acid should always be added slowly to water and in small amounts. Never use hot water and never add water to the acid. Water added to acid can cause uncontrolled boiling and splashing. Sulfuric acid is incompatible with water, bases, organic material, halogens, metal acetylides,

oxides and hydrides, strong oxidizing and reducing agents and many other reactive substances.

4.1.4 Sodium Hydroxide

Sodium hydroxide is a corrosive poison and may be fatal if swallowed. It is harmful if inhaled. Effects from inhalation of mist vary from mild irritation to serious damage of the upper respiratory tract. Contact with skin can cause irritation or severe burns and scarring with greater exposures. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse.

When preparing a solution, always add caustic (pellets or a concentrated solution) to water while stirring, never water to caustic. Sodium hydroxide in contact with acids and organic halogen compounds, especially trichloroethylene, may causes violent reactions. Contact with nitromethane and other similar nitro compounds may cause formation of shock-sensitive salts. Contact with metals such as aluminum, magnesium, tin, and zinc cause formation of flammable hydrogen gas. Sodium hydroxide, even in fairly dilute solution, reacts readily with various sugars to produce carbon monoxide, a poisonous gas that is odorless and colorless.

4.1.5 Sodium Sulfate

Sodium sulfate may be harmful if swallowed and may be irritating to skin, eyes, and mucous membranes. Get medical assistance for all cases of overexposure. If skin is exposed, wash thoroughly with soap and water. If eyes are exposed, immediately flush with water for at least 15 minutes. For dust inhalation, remove to fresh air. For ingestion, if the victim is conscious, have them drink water and induce vomiting immediately as directed by medical personnel. Never give anything by mouth to an unconscious person. Do not heat sodium sulfate in an aluminum container as an explosive reaction may occur.

4.1.6 Acetone

Acetone liquid and vapors are highly flammable. Avoid heat, sparks, open flame, open containers, and poorly ventilated areas when using acetone. Effects of overexposure: acetone is a mild eye and mucous membrane irritant, primary skin irritant, and central nervous system depressant. Acute exposure irritates the eyes and upper respiratory tract. Direct skin contact produces dermatitis, characterized by dryness and erythema through defatting of skin. High concentrations produce narcosis and hypoglycemia.

Emergency first aid – Inhalation: immediately remove to fresh air. If the victim is not breathing, administer mouth-to-mouth rescue breathing. If there is no

pulse, administer CPR. Contact a physician immediately. In case of eye contact, rinse with copious amounts of water for at least 15 minutes. Get emergency medical assistance. Skin contact: flush thoroughly for at least 15 minutes. Wash affected skin with soap and water. Remove contaminated clothing and shoes. Wash clothing before re-use, and discard contaminated shoes. Get emergency medical assistance. Ingestion: call local poison control center for assistance. Contact a physician immediately. Never induce vomiting or give anything by mouth to a victim who is unconscious or having convulsions.

4.2 Equipment and Instruments

Follow the manufacturer's safety instructions whenever performing maintenance or troubleshooting work on equipment or instruments. Unplug the power supply before working on internal instrument components. Use of personal protective equipment may be warranted if physical or chemical hazards are present.

CAUTION: electric shock is a real danger; make sure that the chiller or S-EVAP has no leaks before working around outlets or turning on the heating mantles.

4.3 Pollution Prevention

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA Region 9 Laboratory places pollution prevention as the management option of first choice with regard to environmental management. Whenever feasible, laboratory personnel shall use pollution prevention techniques to address waste generation. When wastes cannot be feasibly reduced, recycling is the next best option. The EPA Region 9 Laboratory *Environmental Management System* provides details regarding efforts to minimize waste.

Minimize waste through the judicious selection of volumes for reagents and standards to prevent the generation of waste due to expiration of excess materials. Reduce the volume of any reagent or standard described in Sections 7.2 or 7.3 so long as good laboratory practices are adhered to regarding the accuracy and precision of the glassware, syringes, and/or analytical balances used to prepare the solution. Reducing the concentration of a reagent is not allowed under this procedure because the impact of such a change on the chemistry of the procedure must be assessed prior to implementation.

Reduce the toxicity of waste by purchasing lower concentration stock standards, lower concentration stock reagents, and solutions to replace neat chemicals whenever

possible. However, do not change the concentrations of standards and reagents specifically designated in this SOP

4.4 Waste Management

The EPA Region 9 Laboratory complies with all applicable rules and regulations in the management of laboratory waste. The laboratory minimizes and controls all releases from hoods and bench operations. All analysts must collect and manage laboratory waste in a manner consistent with EPA Region 9 Laboratory SOP 706, *Laboratory Waste Management Procedure*. Solid and hazardous wastes are disposed of in compliance with hazardous waste identification rules and land disposal restrictions. If additional guidance is needed for new waste streams or changes to existing waste streams, consult with EPA Laboratory Safety, Health, and Environmental Manager (LaSHEM) or ESAT Health and Safety and Environmental Compliance Task Manager or designees.

This procedure generates the following waste streams:

Waste Stream Description	Waste Label	Hazard Properties
Laboratory solid waste (gloves, contaminated paper towels, disposable glassware, etc.)	Non- hazardous Waste	Not applicable
Wastewater with dichloromethane	Hazardous Waste	Corrosive, Toxic
Dichloromethane waste	Hazardous Waste	Toxic
Dichloromethane and hexane waste(applicable for PEST and PCBs)	Hazardous Waste	Flammable, Toxic, contains PCBs
Sulfuric acid and hexane(applicable for PCBs)	Hazardous Waste	Flammable, contains PCBs
Water and hexane(applicable for PCBs)	Hazardous Waste	Flammable, contains PCBs

5 SAMPLE HANDLING AND PRESERVATION

Samples are logged into LIMS and stored in cold rooms maintained at just above freezing to 6 °C in Room 503.

5.1 Containers and Required Sample Volume

In general, water samples should be received in 1 liter bottles containing at least 700 mL of sample. If this is not the case, then the sample volume should be measured and a supervisor notified.

Verify that the integrity of the samples has not been compromised by checking the samples for the following items. Note problems in the LIMS benchsheet and the work order memo field. Specifically note any problems with the following:

- Broken chain-of-custody seals on the sample containers.
- Leaking or broken sample containers.
- Sample information on sample container differing from sample information on chain-of-custody or the LIMS work order. Correct these issues prior to initiating sample extraction through consultation with the supervisor, sample custodian, or EPA Chemistry Team Leader.

5.2 Internal Chain-of-Custody

Verify sample IDs and dates and times of collection against the chain-of-custody form.

Samples are received in Room 503 and maintained under custody. Remove the samples from the walk-in cooler in Room 503 and take them to Room 406 for extraction.

Update the LIMS database internal custody form when sample containers are moved from the designated sample location. Change the container disposition to "active out" and the location to the appropriate room number. At the end of the day, return sample excess samples and empty containers to the "Home" locations. Update the LIMS database using the "return to home location" feature and update container disposition to "available in". Verify that your initials are recorded whenever you update the LIMS custody information.

Verify that the following information on the sample containers corresponds to the information on the tracking sheets and the chain-of-custody record. Any discrepancies must be resolved prior to beginning extraction.

- Client sample ID.
- EPA Region 9 Laboratory ID.
- Case number.
- Sample Delivery Group (SDG) number.

Sample extracts are given by the extraction lab personnel to the appropriate analyst and custody is transferred when the appropriate sections of the completed LIMS benchsheet

are signed by the analyst. Copies of tracking sheets, chain-of-custody records, and the original LIMS benchsheet are to accompany the sample extracts.

5.3 Sample Storage

Samples must be stored at >0 and ≤6°C. Retain samples for 60 days after the final analytical report is sent to the data user.

Label the extracts with the EPA Region 9 Laboratory number and store as follows:

Analysis	Location
SVOCs and 1,4-dioxane,	Room 402 at ≤ -10°C
PEST and PCBs	Room 400 at >0 to ≤6°C
TPH/E	Room 406 at ≤ -10°C

Unless otherwise instructed, retain sample extracts for 40 days (extract holding times expire for all analyses except PCBs).

5.4 Holding Time

Extract samples for SVOCs, SVOCs with 1,4-dioxane, 1,4-dioxane, TPH/E, and organochlorine pesticides within seven days of the time of sampling. If this holding time requirement is not met, notify the EPA Chemistry Technical Director so that a course of action can be determined. PCBs samples do not have a holding time recommended in the method. If the extraction date is over one year after the sampling date, put a note in the LIMS work order memo field; do not qualify the data.

6 INTERFERENCES

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to anomalous peaks or elevated baselines in gas chromatograms.

Contamination of clean glassware, apparatus, and instruments and may occur from processing samples containing high levels of contaminants, especially PCBs. Exhaustive clean up of affected items may be required to eliminate the contamination.

Phthalate esters are commonly used as plasticizers and are easily extracted from plastic materials. Contact of samples, solvents, reagents, glassware, extracts, or other sample processing apparatus with plastics must be avoided.

Elemental sulfur in sample extracts can interfere with the chromatographic determination of certain analytes, especially pesticides. Sulfur can be removed by the use of copper or GPC cleanup. See USEPA Region 9 Laboratory SOP 260 for details on GPC cleanup procedure.

7 APPARATUS AND MATERIALS

This section describes recommended apparatus and materials to be used for the analysis. All equipment, reagents, standards, and supplies must meet the technical and QC requirements of the reference method. Substitutions may be made provided that they are documented and equivalency is maintained.

7.1 Instruments and Equipment

- Continuous Liquid-Liquid Extractor (CLLE) Apparatus, (VWR part # 89091-206) or equivalent.

  Continuous Liquid-Liquid Extractor – with glass connecting lines for use with dichloromethane (Hershberg-Wolf Extractor, Ace Glass Company, Vineland, NH, P/N 6841-10 or equivalent).

  Condenser, Allihn with 45/50 lower joint – Supelco, or equivalent.

  Heating Mantle – Electrothermal, ET1000/C, or equivalent.

- Heating/Refrigerated Recirculating Chiller – 0 - 40°C temperature range, 3.8 L/min flow at 60 psi, 750 watt cooling capacity at 20°C, VWR model 1172 or equivalent.

- Round Bottom Flasks – 500-mL, and 1,000-mL.

- Kuderna-Danish (K-D) Apparatus

  Concentrator Tube – 10 mL, graduated (Kontes K-570040-1029, or equivalent)

  Evaporative Flask – 500 mL (Kontes K-470001-0500, or equivalent)

  Snyder Column – three-ball macro (Kontes K-503000-0121, or equivalent). The ideal overall length is 23-29 cm (9-11.5 in.); optimal length is 26 cm (10 in.).

- Analytical balance – 2000 gram capacity and capable of weighing accurately to ± 1 g, (Mettler top loading balance, or equivalent).

- Solvent Evaporation Device (S-EVAP™) – combination water bath and solvent recovery system (S-EVAP™, Organomation Model 120, or equivalent).

Alternatively, water bath (heated, with concentric ring cover, capable of temperature control with stability between 60°C - 100°C) and solvent recovery manifold.

NOTE: The S-EVAP™ or water bath must be located under a fume hood.

- Nitrogen Evaporation Device – with the capability of temperature control in a heated dry media bath (N-EVAP™, Organomation Model 111, or equivalent).

- Apparatus for eluting Florisil cartridges – Vacuum manifold system to include glass chamber with vacuum valve and top plate with flow control valves. Restek ResprepJ-12T Catalog No. 24001 or equivalent.

- Funnels and Boiling Chips

  Funnels – 16 cm ID, long stem

  Teflon Boiling chips – prepare by transferring boiling chips to a Soxhlet extractor with approximately 300 mL of dichloromethane. Reflux for 1-2 hours.

- Miscellaneous Items

  Graduated Cylinders – 10-mL, 100-mL, and 1- L

  Syringes, gas-tight style, various sizes (depending on final volume) including: 50-uL, 100-uL, 500-uL, 1-mL, 2.5-mL, 5-mL, and 10-mL.

  Vortex Mixer or Sonicator

7.2 Reagents

Document the receipt and preparation of all reagents in the LIMS.

Reagents may contain impurities that might affect analytical data. Use only reagents that conform to the American Chemical Society (ACS) reagent grade specifications or better. If the purity of a reagent is in question, analyze for contamination prior to use.

- Acetone – capillary GC/GC-MS solvent grade

  Caution: Acetone is highly flammable. See Section 4.1.6 for precautions.

- Dichloromethane – Recycled and graded or pesticide residue grade, or equivalent See USEPA Region 9 Laboratory SOP 239, *Recycling Used Methylene Chloride*. There are four grades of recycled dichloromethane: Pesticide & PCBs, BNA, TPH

and Rinse Only. Use only the appropriate grade of dichloromethane for each analysis or task.

Caution: Dichloromethane is a suspected carcinogen. See Section 4.1.1 for precautions

- Hexane – capillary GC/GC-MS solvent grade suitable for pesticide residue analysis. Caution: Hexane is extremely flammable. See Section 4.1.2 for precautions.

- Hexane: acetone (9:1 v/v) – prepare immediately prior to use in the Florisil cleanup procedure for PEST extracts. The volume prepared is based on the number of Florisil cartridges to be eluted –about 25 mL per cartridge is sufficient.

- Nitrogen – house nitrogen from liquid nitrogen at approximately 150 psig

- Glass wool (Ohio Valley Specialty Chem. cat. No. 3350 untreated), rinse with dichloromethane and dry in hood prior to use. Assign a "rinse date" and record in benchsheet when used.

- Reagent water– water which is free of interferences at or above one-half the analyte reporting limits and used in preparation of solutions requiring the use of water for all reagent blanks.

- Sodium hydroxide (NaOH) – 10N. Dissolve 40 g NaOH in reagent water and dilute to 100 mL. Sodium hydroxide solutions are corrosive. See Section 4.1.4 or precautions.

- Sodium sulfate – granular, anhydrous reagent grade

  NOTE: Before use, heat at 400°C for 4 hours, or at 120°C for 16 hours, cool in a desiccator, and store in a glass bottle.

  Caution: do not use an aluminum container to heat sodium sulfate as it may result in an explosive reaction at high temperatures.

- Sulfuric acid – reagent grade

  Caution: Sulfuric acid is extremely corrosive. See Section 4.1.3 for precautions. Sulfuric acid is stored in Room 501.

- Sulfuric Acid (H_2SO_4) – 1:1 v/v. Slowly add 50 mL of H_2SO_4 to 50 mL of reagent water. Perform dilution in a fume hood.

Caution: Sulfuric acid is corrosive. Extreme caution must be exercised as both heat and acid fumes may be generated by this process. Never add water to sulfuric acid. See Section 4.1.3.

7.3 Standards

Document the receipt and preparation of all standards in the LIMS database.

Opened ampules and working standards must be maintained in a freezer at ≤-10 °C and protected from light. The expiration date of the standard solution is either 6 months from preparation date or the expiration date of the stock standard used in the preparation, whichever is earlier. Analysts must allow all standard solutions to equilibrate to room temperature before use.

- Florisil Cartridge Check Solution – A solution of 2,4,5-trichlorophenol in acetone at a concentration of 0.1 ug/mL (Restek 32017-500 or equivalent).

- Florisil Pesticide Check Solution – A solution of organochlorine pesticides at 0.02/0.04/0.20 ug/L and surrogates at 0.08 ug/L that is used to check Florisil cartridge recovery of analytes. Prepared from commercial mixture such as Restek Organochlorine Pesticide Mix AB#2, catalog No. 32292 and Restek Pesticide Surrogate Mix, catalog No. 32000 or equivalent.

- Matrix Fortifying Solution – Use the matrix fortifying solutions listed in the following table to add to each LCS and MS/MSD sample prior to extraction.

Table 7.1

Analytes	**Conc., µg/mL**	**Vol. to add, µL**
PEST	0.4/0.8/4.0	500
Semivolatile Organics and 1,4-Dioxane	20/100	500
PCBs	2	500
TPH/E	3,000	500

- Surrogate Analyte Solution - Add surrogate analyte solution to each field and QC sample prior to extraction. The following table lists the surrogates and the amounts to add for each method.

Table 7.2

Analytes	Conc., µg/mL	Vol. to add, µL
PEST	0.4	500
Semivolatile Organics (w/ 1,4-dioxane d8)	10/100/150	500
PCBs (use PEST mix)	0.4	500
TPH/E	300	500

Instructions for preparing the above solutions are provided in EPA Region 9 Laboratory SOP 290, Appendix E.

7.4 Supplies

- Final Volume Containers (will vary according to analysis)

  Screw-top vials 2.0ml (Supelco part No. 2-7329 or equivalent)

  ABC Screw Cap-PTFE/Silicone/PTFE Septa (Supelco part No. 27560-U or equivalent)

  Screw-cap vials 5.0ml (Kimble part No. 60910 or equivalent)

  Caps 5ml- Teflon liner (Wheaton part. No. 240408/ VWR# 66012-350)

  Screw thread vials 10.0ml (Kimble part No. 60910-3)

  Caps 10ml- Teflon liner (Wheaton part. No. 240409/VWR# 66012-372)

- Vials 40ml (VWR cat. No. 15900-034)
- Pasteur pipettes – disposable (VWR cat No. 14672-380)
- Pipette, disposable – 1.0-mL, 5.0-mL, 10.0-mL
- Pipet Bulb (VWR cat No. 56311-062)
- Kimwipes EX-L (Kimberly-Clark VWR code 34155 11 X 21 cm)
- Aluminum Foil, heavy duty
- pH Paper – wide range 0-14 (EM Science cat. No. 9590)

- Florisil cartridges – 1 g cartridges with stainless steel or Teflon frits. Supelco ENVI-FLORISIL Catalog No. 57053 or equivalent

8 ANALYTICAL PROCEDURES

The LIMS must be used as an integral part of the extraction process. Review the project notes, work order memo field, sample notes, COC and other documentation for project or sample specific instructions. Check for samples designated by the client for MS/MSD. Prepare all client-requested QC. If no requests are indicated on the COCs, prepare one MS/MSD per SDG of 20 or fewer samples. Organize samples into batches; complete benchsheets as the work progresses. Note that samples for SVOC, SVOC with 1,4-dioxane, and 1,4-Dioxane can be batched together. Enter the LIMS IDs for all reagents (acids, bases, solvent, sodium sulfate, etc.) into the batch reagent section and the spiking and surrogate solution in the benchsheet. Use batch notes to record other information such as the start and stop dates and times for the CLLEs and the rinse date for the glass wool, if it is used.

Export the benchsheet to Excel; enter weights into the information fields directly from the balance software; and import the spreadsheet back into LIMS which will calculate initial volumes. The benchsheet must be printed in a layout that includes the information fields. Alternatively, if either LIMS or the balance software is unavailable, record full and empty sample bottle weights in the Comments column of a printed copy of LIMS benchsheets. This annotated benchsheet must accompany the sample extracts. See Section 8.3.1.

Note: The procedures for extracting SVOCs, SVOCs with 1,4-dioxane, and 1,4-Dioxane are identical. For simplicity, the following instructions use the terms "SVOCs" or "semivolatile analyses" to encompass these analyses.

8.1 Instrument Operation

8.1.1 Refrigerated recirculator

Turn on the heated/ refrigerated recirculator(s); these supply cold water to the CLLE Allihn condensers. They are located in the service corridor behind room 404. Check the water level and the system for leaks; do not proceed until all leaks are fixed. Add water as necessary. Verify that the temperature is set at 15°C and the pressure is about 15 psi.

Caution: This step is essential to minimize dichloromethane exposure.

8.1.2 S-EVAP™

Turn on S-EVAP™ when the sample extracts are ready for concentration. Check the water level; it should be up to or just below drain (located inside water bath). Add water as necessary and adjust the S-EVAP™ heat control to

between 85-90°C for dichloromethane and 90-95°C for hexane exchange; allow about 45 minutes for the water bath to warm up.

Turn on cold water connected to S-EVAP™ condenser and adjust flow to at least 1,000 mL/min. Check system for leaks; stop immediately and fix all leaks before proceeding.

8.1.3 N-EVAP™

Turn the N-EVAP™ on when the sample extracts are ready for concentration and adjust the dry media bath temperature to 2-5°C below the boiling point of the solvent (34°-37° C for dichloromethane and 64°-67° C for hexane); if possible, measure the dry media bath temperature where the concentrator tubes are placed in the dry media bath. Clean N-EVAP™ needles with solvent before use.

Set the concentrator tubes with the sample extracts in the dry media bed in a secure position. At least 1" of the concentrator tube should be below the surface of the dry media. Place the needle inside the concentrator tube without contacting the solvent or the concentrator tube itself.

8.2 Preparation for Extraction

Query the backlog using LIMS to determine which class of contaminants must be extracted (SVOCs including 1,4-dioxane, PEST, PCBs or TPH/E). Use the extraction holding time date to determine what work must be completed first. Select and batch the appropriate samples. Follow the procedures listed in Section 5.2 to retrieve the corresponding samples. Record problems in the comments section of the benchsheet or LIMS work order memo field; contact the Organics Group Leader, if appropriate.

Select sufficient glassware for the number of samples and QC samples in the batch. Place the apparatus (round bottom flask and extractor body) in the hood with the extractor body resting on the platform in the fume hood and the round bottom flask on a cork ring.

Triple rinse the CLLE extractor and round bottom flask with recycled dichloromethane. Select 500-mL or 1,000-mL round bottom flasks depending upon the heating mantles you will be using for the extraction. Collect the rinsate for recycling.

Add two to four Teflon boiling chips to the round bottom flask and assemble the CLLE apparatus. Label the flask with the laboratory sample ID and the type of sample (i.e. MB, LCS, MS/MSD).

Add approximately 300 mL of dichloromethane to the extractors. Dichloromethane will flow into the round bottom flasks from the extractor bodies.

Sort bottles by date sampled so that samples can be analyzed chronologically according to sample date (not date received) to prevent exceeding the extraction holding time.

8.3 Sample Extraction

Let sample bottles sit on counter until sample nears room temperature then wipe condensation off.

Export the benchsheet to Excel and use a top loading balance and record the weight of each sample container with sample in the information column of the LIMS benchsheet.

> NOTE: The sample density must be nearly 1.0 for the assumption that sample weight equals sample volume to be true. An empty 1-L amber bottle of the type generally used for sampling weighs approximately 500 grams. When "full" the bottle should weigh 1500 to 1550 grams. Full bottles which weigh more or less than this indicate that the density is not 1.0 and the sample may not be aqueous. Adjust the expected weight if the bottle is not nearly full using the density of 1 gram per milliter to calculate an expected weight. The analyst must seek guidance if the apparent density of the sample is not 1.0. (Several test may be necessary to determine if the sample can be extracted with dichloromethane – for example, materials which are misible must be analyzed using the waste dilution method. At a minimum, the actual volume of sample must be measured by marking the meniscus on the bottle, emptying the container as usual, rinsing per the following instructions, and then filling the bottle with water to the mark and using a graduated cylinder to determine the sample volume).

Fill a large graduated cylinder with deionized water to the 1,000-mL mark. Record the volume in the LIMS benchsheet and pH in the benchsheet comments; pour MB QC sample into an extractor body. Repeat for LCS.

Mix each sample thoroughly by inverting the sample container several times.

Open sample container and pour sample into the CLLE extractor body. Use a pH strip to measure the pH of the sample while pouring.

Rinse the sample container with three portions of approximately 20 mL each of dichloromethane and transfer this rinsate to the extractor.

Record the pH and the weight of the empty sample bottle in the appropriate columns of the Excel spreadsheet.

NOTE: If a sample weighs less than 700 g, add reagent water to increase sample weight to approximately 1,000g. This SOP assumes that the density of water is 1.0; therefore 1 gram = 1 mL of water. Record the actual volume of sample extracted in the LIMS benchsheet.

MS/MSD QC samples are prepared the same way as field samples, pH is recorded in the LIMS benchsheet comment column. The MS/MSD concentration is used to calculate %RPD; therefore, it is imperative to extract equal volumes. It may be necessary to split one bottle between two extractors if the samplers did not provide sufficient volume. Be certain to place equal volume in each extractor and to carefully split the bottle rinsate between the MS and MSD.

NOTE: The best way to achieve equal MS/MSD sample size is to weigh a full sample bottle and then to subtract the weight of an empty bottle, empty bottles will not vary in weight by more than a gram or two, this will give the approximate size of the sample. Divide the result in half and add it to the weight of the empty bottle; this is the target weight. Now pour the MS sample slowly into an extractor body, checking the weight of the sample bottle frequently while doing so. Stop when you reach target weight. Pour rest of sample into separate extractor body for the MSD and rinse bottle with solvent as normal and pour rinsate into both extractor bodies. Calculate the sample size of the MS & MSD and add deionized water as necessary.

For example: sample bottle weight (full) = 1450g, sample bottle weight (empty)=500g

1450g-500g=950g or approximate sample weight

950g/2=475g + 500g (empty bottle)=975g (target weight)

pH adjustment – Adjust the sample pH to the extraction pH using 1 mL aliquots of 1:1 v/v sulfuric acid or 10N sodium hydroxide if required. Stir the sample after each addition to mix acid or base into sample with a disposable Pasteur pipette or a long glass stirring rod (rinse with dichloromethane before inserting into extractor). Allow the tip of the pipette or rod to come in contact with pH paper to determine the pH of the sample. Record the extraction pH in the LIMS benchsheet.

SVOC and 1,4-Dioxane Samples – Adjust the pH of the sample to <2 by adding 1-3mL of 1:1 v/v sulfuric acid to the sample in the extractor body. Make sure not to over-acidify the sample.

PEST and PCBs Samples – Adjust the pH of the sample to between 5 and 9 by adding either 1:1 v/v sulfuric acid or 10N sodium hydroxide to the sample in the extractor body. DO NOT adjust the pH of the method blank, LCS or PT samples prepared in deionized water for PEST – pH measurements of deionized water are

unreliable and adjusting the pH, even with a drop of 10N sodium hydroxide, has been shown to result in the loss of Endosulfan I and II.

TPH/E Samples - Adjust the pH of the sample to <2 by adding 1-3mL of 1:1 v/v sulfuric acid solution to the sample in the extractor body.

Allow surrogate and matrix spike solutions to come to room temperature before using. Re-mix the solutions by agitating them manually, or with a Sonicator or a Vortex mixer. Make sure that the solutions do not contain any precipitate. This is important to ensure acceptable surrogate and spike recovery.

Add the appropriate surrogate solution to each sample (see Table 7.2).

Add the appropriate matrix spike solution to the QC samples labeled MS, MSD, and LCS (see Table 7.1).

Take the CLLE apparatus (extractor body and round bottom flask) from the hood and place the extractor body with flask in the heating mantle and clamp the extractor body with the cylinder clamp.

Place the Allihn condenser on top of the CLLE extractor body.

Turn on the heated/ refrigerated recirculator(s). These are located in the service corridor behind room 404. Check the water level in the recirculators; add deionized water and algacide as necessary. Verify that the temperature is set at 15°C and the pressure is about 15 psi.

Caution: This step is essential to minimize dichloromethane exposure.

Wait 15 minutes then turn on the heating mantles to level 4 and record the start time and date in the LIMS batch. Check the samples within 15 minutes to ensure that the drip rate of solvent into the extractor body has reached a rapid drip, but is not a steady stream. You should be able to distinguish drops, but not count them. If you can count the individual drops of solvent the heating rate is insufficient. If you observe a steady stream of solvent, the heating rate is too high. Adjust the heating mantles as necessary. In addition, the solvent must drip directly into the sample, not down the side of the glass. These adjustments are important to achieve proper extraction.

Extract the samples for 18 – 24 hours.

Turn the heating mantle off after the extraction is complete and record the stop time and date in the LIMS batch. Keep the refrigerated chiller running for 45 minutes to let the flasks cool.

Caution: Do not remove the condensers or separate any of the parts of the extractor apparatus during this cooling period. This is important to prevent exposure to dichloromethane.

Before removing the flask from the CLLE apparatus, tilt the CLLE apparatus to drain most of the dichloromethane in the extractor body into the flask. Do not allow water to drain into the flask. Remove the flask from the extractor. Discard the water layer in the laboratory liquid waste container. If more than a milliliter or two of water drains into the flask, pour the extract into a separatory funnel to partition the phases.

NOTE: If the sample is not to be concentrated immediately, cover it with aluminum foil and store in freezer unit in Room 406 at $\leq$-10°C.

8.3.1 Determination of Initial Volume

The Excel spreadsheet completes this calculation so simply import the completed spreadsheet to the LIMS benchsheet. (If weights were recorded by hand, subtract empty sample bottle weight from full sample bottle weight located in comments section of LIMS benchsheet. This is sample weight. Assume a density of 1.00g/mL for water samples so weight equals volume. Enter the volume in "Initial" column of benchsheet in LIMS.)

8.4 Extract drying

Residual water must be removed from all sample extracts before proceeding.

- Prepare a long-stemmed funnel for use as a drying tube by placing a 0.25-0.5 inch plug of glass wool in the top portion of the stem. Add approximately 20 to 30 g of anhydrous sodium sulfate (Na_2SO_4); use more for wet extracts.

- Rinse the prepared drying tube with approximately 20 mL of dichloromethane. Discard rinsate in the appropriate waste solvent container.

- Collect the filtrate in a 500-mL K-D flask fitted with a concentrator tube. Quantitatively transfer the contents of each round bottom flask into a drying tube (see above). Rinse the round bottom flask three times with approximately 20 mL of dichloromethane. Add the rinsates to the funnel and collect in the K-D flask. Rinse the funnel three more times.

8.5 Concentration

8.5.1 Concentration with K-D apparatus

Make sure the S-EVAP™ has reached the appropriate temperature and has adequate flow; see Section 8.1.2.

Add 3-4 boiling chips to the K-D. Place a three-ball Snyder column on the K-D flask. Wet the Snyder column by squirting about 2 mL of dichloromethane from a Teflon wash bottle into the top of the column.

Place the assembled K-D in one of the positions on the S-EVAP™ and connect the Snyder column to the condenser connected to the central solvent collector.

- For semivolatiles – evaporate the solvent to an apparent volume of 2-5 mL. Do not allow the extract to go to dryness. Remove the K-D apparatus (concentrator tube, K-D flask, and Snyder column) from the S-EVAP™ and cover the end of the Snyder column with aluminum foil. Wipe or wrap the joint between the concentrator tube and K-D flask with several Kimwipes or a paper towel to prevent water from getting into the extract. Set aside for 5 to 10 minutes to cool and to allow the remaining solvent to drain from the Snyder column into the concentrator tube. The volume will increase to approximately 5-7 mL after the solvent in the apparatus cools. Wipe the outside of the concentrator tube and K-D flask thoroughly before disconnecting the two pieces to prevent contamination of the sample extract.

- For PEST and PCBs – evaporate the solvent to an apparent volume of 10 to 15 mL then disconnect the S-EVAP™ condenser from the Snyder column. Empty the dichloromethane from the S-EVAP™ collection flask for recycling and reconnect the flask. Add 15-20 mL of hexane to the top of the Snyder column and allow it to wash down to the K-D flask; then add another 15-20 mL of hexane to the top of the Snyder column. Replace the S-EVAP™ condenser on the Snyder column and evaporate the solvent to an apparent volume of 2-5 mL. Do not allow the extract to go dry. Remove the K-D apparatus (concentrator tube, K-D flask, and Snyder column) from the S-EVAP™ and cover the end of the Snyder column with aluminum foil. Wipe or wrap the joint between the concentrator tube and K-D flask with several Kimwipes or a paper towel and set aside for 5 to 10 minutes to cool and to allow the remaining solvent to drain from the Snyder column into the concentrator tube. Spin the K-D to wash the solvent over the glass and make certain that no analyte has adhered to the glass surface. The volume will increase to approximately 5-7 mL. Wipe the outside of the concentrator tube and K-D flask thoroughly before disconnecting the two pieces to prevent contamination of the sample extract. Empty the contents of the S-EVAP™ collection flask into the dichloromethane/hexane waste container.

- For TPH/E – evaporate the solvent to an apparent volume of just under 5 mL. Do not allow the extract to go dry. Remove the K-D apparatus (concentrator tube, K-D flask, and Snyder column) from the S-EVAP™ and cover the end of the Snyder column with aluminum foil. Wipe or wrap the joint between the concentrator tube and K-D flask with several Kimwipes or a paper towel and set aside for 5 to 10 minutes to cool and to allow the remaining solvent to drain from the Snyder column into the concentrator tube. The volume will increase to approximately 5-7 mL after the solvent in the apparatus cools. Wipe the outside of the concentrator tube and K-D flask thoroughly before disconnecting the two pieces to prevent contamination of the sample extract.

Turn the S-EVAP™ heat control to OFF. Turn off the cooling water to the S-EVAP™ system.

Empty the solvent collected in the central solvent collection system of the S-EVAP™ into the appropriate waste container.

8.5.2 Concentration with Nitrogen Evaporation apparatus

Use a nitrogen evaporator (N-EVAP™) to concentrate the extract to the required final volume. Clean N-EVAP™ needles before use with same solvent as the sample extract. Adjust the dry media bath temperature to about 2-5°C below the boiling point of the solvent (see table below); if possible, measure the dry media bath temperature where the concentrator tubes are placed in the dry media bath. Set the concentrator tubes with the sample extracts in the dry media bed in a secure position. At least 1" of the concentrator tube should be below the surface of the dry media. Place the needle inside the concentrator tube without contacting the solvent or the concentrator tube itself.

Dry Media Bath Conditions

Solvent	Boiling point, °C	N-EVAP™SET POINT, °C	Acceptable range, °C
Dichloromethane	39	37	34-37
Hexane	68.7	67	64-67

Verify that the valves on the N-EVAP™ manifold are closed and set the needle valve controlling the nitrogen flow to the N-EVAP™ manifold OFF. Open the nitrogen valve on the left side of the hood and control the gas flow going into the tube using the needle valve and the valve on the N-EVAP™ manifold.

Maintain a gentle nitrogen flow and monitor it frequently to ensure the solvent does not splash and that the tube does not go dry.

Final volume is dependent upon the class of contaminants:

Analyte	Final Solvent	Evaporation Volume, mL	Final Volume, mL
PEST and PCBs	Hexane	5	10.0
Semivolatile Organics	Dichloromethane	0.7	1.0
TPH/E	Dichloromethane	2	3.0

- For semivolatiles – evaporate the extract to approximately 0.7 mL. Draw the extract into a clean 1-mL syringe. Using the extract in the syringe, rinse the sides of the concentrator tube, and draw the extract back into the syringe. Rinse down the sides of the concentrator tube with approximately 250 µL of dichloromethane and draw this rinsate into the 1-mL syringe containing the sample extract. Add a small amount of dichloromethane to the concentrator tube and adjust the volume in the syringe to the final extract volume of 1.0-mL with this solvent.

  Transfer the extract to a Teflon-sealed screw-cap bottle labeled with laboratory assigned sample number and the type of sample (BNA), and store at ≤ -10°C in Room 406. The extract is now ready for SVOC GC/MS analysis.

- For PEST and PCBs – evaporate the extract volume to approximately 5 mL. Draw the extract from the concentrator tube into a 10.0-mL syringe. Rinse the concentrator tube three times with approximately 1 mL each time of hexane and draw these rinsates into the 10.0-mL syringe containing the sample extract. Add approximately 2 mL of hexane to the concentrator tube and adjust the volume in the syringe to the final extract volume of 10.0 mL with this solvent.

  Transfer the extract to a 10-mL Teflon-sealed screw-cap bottle labeled with the laboratory assigned sample number and the type of sample (PEST, PCBs, etc.) and store at >0°C - ≤6°C in Room 400. The extract is now ready for PEST analysis unless a Florisil clean-up is required; see Section 8.5.4. If a Florisil clean up is required, keep 3.0 mL of sample in reserve and clean up 2.0 mL. If Florisil clean up is not required, provide 5.0 mL to the analyst.

For PCBs, perform acid cleanup according to Section 8.5.3.

- For TPH/E – evaporate the extract volume to 2.0 mL. Draw the extract from the concentrator tube into a 5.0-mL syringe. Rinse the concentrator tube three times with approximately 250 µL each time of dichloromethane and draw the rinsates into the 5.0-mL syringe containing the sample extract. Add a small amount of dichloromethane to the concentrator tube and adjust the volume in the syringe to a final extract volume of 3.0 mL with this solvent. Transfer the extract to a Teflon-sealed screw-cap bottle labeled with the laboratory assigned sample number and the type of sample (TPH/E) and store at ≤-10° in Room 406. The extract is now ready for analysis for TPH/E.

NOTEs:
(1) Do not allow the concentrator tube to go dry during the concentration step.

(2) The tube should be cool as it is rinsed or analyte loss will result.

(3) Adjust final volume very carefully as a small error will result in a large analytical error.

(4) IF THE VOLUME IN THE SYRINGE ACCIDENTALLY EXCEEDS THE REQUIRED FINAL VOLUME, RETURN THE EXTRACT TO THE CONCENTRATOR TUBE AND BEGIN THE EVAPORATION PROCESS AGAIN.

Close the N-EVAP™ manifold valve when concentration step is complete.

8.5.3 Acid Cleanup for PCBs Extracts

Transfer approximately 5 mL of the extract (now in hexane) to a 40 mL vial and very slowly add 5 mL of concentrated sulfuric acid. Store the remaining sample extract at >0 to ≤6°C in the refrigerator in Room 406.

Vortex 1 minute (vortex must be visible in the extract) and allow phases to separate for 5 to 10 minutes. If the hexane (top) layer is not colored or cloudy and does not form a visible emulsion, proceed. If the hexane layer is cloudy, transfer top (hexane) layer to a clean 40-mL vial, very slowly add 5 mL of concentrated sulfuric acid and vortex. Should the extract remain colored or cloudy or form an emulsion, seek assistance from the Organic Group Leader. Discard the acid (bottom) layer in acid waste container.

Transfer 3 to 4 mL of the hexane layer to a Teflon-sealed screw-cap bottle labeled with the laboratory assigned sample number and the type of sample. Exercise caution to avoid drawing acid along with the hexane because any acid in the extract will damage or destroy instrument components. A cloudy extract

may also contain acid, so do not proceed until the situation is resolved. Once the extract is clear, add approximately 200 mg of sodium sulfate to the vial and cap.

Store at >0°C - ≤6°C in Room 400. The extract is now ready for analysis for PCBs.

8.5.4 Florisil Cartridge Cleanup for PEST extracts

Inspect the color and clarity of the extracts to determine if they may require Florisil cleanup. If the extracts are clear and colorless, analyze a typical extract. If no chromatographic interferences are present, analyze the samples without Florisil cleanup. If the samples are colored or if interferences are present, proceed with Florisil cleanup.

Connect the vacuum manifold to house vacuum; install a trap (2-L Erlenmeyer vacuum flask wrapped with tape) between the vacuum manifold and house vacuum.

Florisil cartridges must be washed with hexane:acetone (9:1 v/v) before use. Place a Florisil cartridge into a flow control valve on the vacuum manifold for each sample extract to be processed; place a waste collection container below each Florisil cartridge.

Pass at least 6 mL of the hexane:acetone solution through each cartridge; use vacuum to elute the cartridges if necessary. Allow most of the solvent to pass through the cartridge filter. Close the cartridge flow control valve when there is a thin layer of solvent above the top of the cartridge filter. Release the vacuum and remove the waste collection container; discard the rinsate in appropriate waste container.

DO NOT ALLOW CARTRIDGE FILTERS TO GO DRY AFTER THEY HAVE BEEN WASHED. If a cartridge filter goes dry before the addition of the extract, discard it and begin again.

Place labeled collection vials for each Florisil cartridge inside the vacuum manifold. Make sure that the solvent line from each cartridge is placed inside the appropriate vial as the manifold top is replaced.

Transfer a 2.0 mL aliquot of each field or QC sample extract to the top frit of the appropriate Florisil cartridge. Store the remaining sample extract at >0 to ≤6°C in the refrigerator in Room 406.

Restore the vacuum and open the cartridge flow control valve; allow most of the extract aliquot to pass through the cartridge filter. Close the cartridge flow

control valve when there is a thin layer of solution above the top of the cartridge filter. If a cartridge goes dry after the extract has been added, discard it and take another 2 mL of sample extract out of the reserve and begin again.

Open the cartridge flow control valve and elute the PEST in the extracts from the Florisil cartridge with 9.0 mL of hexane:acetone (9:1) v/v. Use vacuum to elute the cartridges if necessary.

Concentrate the extract to the same 2.0 mL volume as was taken for cleanup using the nitrogen evaporator (N-EVAP™) as described in Section 8.5.2 for organochlorine pesticides. Transfer the extract to a Teflon-sealed screw-cap bottle labeled with the laboratory assigned sample number and the type of sample and store at >0°C to ≤6°C in Room 400. Record the final volume as 10 mL.

The extracts are now ready for analysis for organochlorine pesticides.

8.6 Maintenance

Heating/Refrigerated Recirculating Chiller

- Check water level and add deionized water if necessary before turning on.
- Take out and inspect the chiller filter periodically (monthly), clean filter when needed.
- Add capful of bleach or algaecide to circulating water if water looks greenish or black.
- Check chiller temperature and flow rate periodically while instrument is running to make sure they can maintain appropriate settings.

Nitrogen Evaporation Device (N-EVAP™)

- Check that all silicon tubing is in good shape and connected to all needle valves before using.
- Verify that there are enough aluminum beads in the dry media bath to come to ~1/2 inch from the top; if not, add as necessary.
- Clean needles with solvent before use.

- If the gas flow rate is too slow or too great, inspect the needle valve to make sure it is opened to the appropriate amount. If flow rate problems still occur, check the nitrogen tank located in the EMFAC area; change the tank if necessary.

Solvent Evaporation Device (S-EVAP™)

- Check bath water level, fill if necessary.
- Check accuracy of heating unit annually; should be within 2°C of digital display temperature.
- Periodically inspect unit for leaks and cracks in glass fittings.
- Change bath water if water looks greenish or black; this is usually not a problem if drain is not plugged.
- Check that water flows properly and there are no plugs and that all tubing runs into drain in hood.

Additional maintenance requirements are provided in Appendix D.

9 QUALITY CONTROL

The EPA Region 9 Laboratory operates a formal quality control program. As it relates to this SOP, the QC program consists of a demonstration of capability, and the periodic analysis of MB, LCS, and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated. A summary of QC criteria is provided in Appendix C of the applicable analytical SOP.

9.1 Demonstration of Capability

A Demonstration of Capability must be in place prior to using an analytical procedure and repeated if there is a change in instrument type, personnel, or method. Follow procedures described in EPA Region 9 Laboratory SOP 880.

9.2 Batch QC

Assessment of QC sample results is done under USEPA Region 9 Laboratory SOPs 315, 330, 335, and 385 where frequency, QC acceptance criteria, and corrective action are defined.

9.2.1 Method Blank

Method Blanks are used to determine the level of contamination introduced by the laboratory during the extraction procedure. The blanks are subjected to the same extraction and cleanup procedures that are used for samples. One MB is extracted with each extraction batch.

9.2.2 Laboratory Control Sample

The LCS is a MB spiked with matrix fortifying solution. The matrix fortifying compounds are used as indicators of extraction efficiency in the absence of matrix interferences. One LCS is extracted with each group of twenty or fewer samples extracted together.

9.2.3 Matrix Spike Sample Matrix and Matrix Spike Duplicate

MS and MSD analyses provide information about the effect of the sample matrix on sample preparation and measurement. Poor %R results and large RPD between duplicates may indicate inconsistent laboratory technique, sample non-homogeneity, or matrix effects which may interfere with analysis. A solution of matrix fortifying compounds is spiked into the QC samples designated by the samplers or the sample custodian. A set of MS/MSD samples is prepared with each sample delivery group of twenty or fewer samples.

9.3 Sample QC

Each field and QC sample is fortified with a surrogate solution prior to extraction. Surrogate %R provides information about both the laboratory performance on individual samples and the possible effects of the sample matrix on the analytical results.

9.4 Florisil Cartridge Performance Check

Each lot number of Florisil cartridges must be tested before they are used for sample cleanup. Follow procedures outlined in Appendix B.

The lot of Florisil cartridges is acceptable if all pesticides are recovered at 80-120%, the recovery of 2,4,5-trichlorophenol is less than 5% (compound should be retained on Florisil cartridge), and no peaks interfering with the target analytes are detected.

10 DOCUMENTATION

10.1 Bench Sheet

Complete a LIMS benchsheet and for each extraction batch. Submit a copy to the analyst with the sample extracts. This copy is included in the data package. Make sure all information requested on the benchsheet is completed fully (i.e. dates, amounts, reagents, start and stop time for CLLEs, initials, and comments) before delivering to the analyst who signs as accepting the batch after peer reviewing the entries.

10.2 Standards

All standards (Surrogate, MS/MSD, and LCS spiking solutions) are recorded in the LIMS. A copy of each Analytical Standard Record associated with sample analysis must be included in the data package.

10.3 Reagents

Record all reagents used for each analytical batch in the LIMS and on the benchsheet or batch.

10.4 Maintenance Logbook

Maintain a maintenance logbook for each instrument covered in this SOP. Document the following:

- Initial installation and performance
- Subsequent instrument modifications and upgrades, including major software upgrades
- All preventive or routine maintenance performed including repairs and corrective or remedial actions. Whenever corrective action is taken, record the date, the problem and resolution, and documentation of return to control.

All entries should be made in accordance with EPA Region 9 Laboratory SOP 840, *Notebook Documentation and Control.*

10.5 SOP Distribution and Acknowledgement

After approval, distribute an electronic copy of the final SOP to all laboratory staff expected to perform the SOP or review data generated by the SOP. (The Lab QC Database contains a list of assigned analysts for each SOP). All approved EPA

Region 9 Laboratory SOPs are maintained in the LotusNotes database in Adobe Acrobat portable document format.

Analyst training is documented via the Training Record form and the Read and Understood Signature log; the latter is entered into the Lab QC Database.

10.6 SOP Revisions

Revisions to this SOP are summarized in Appendix E.

11 REFERENCES

Instruction Manual for S-EVAP™ Model 120 Solvent Evaporation System, Organomation Associates Inc.

Instruction Manual for N-EVAP™ Models 111,112,115 Nitrogen Evaporation System, Organomation Associates Inc.

Operators Manual for Refrigerated Recirculating Chillers Model 6306, Polyscience, Document No. 110-266, Revision C, February 2004

U.S. Environmental Protection Agency SW-846 Method 3500B, *Organic Extraction and Sample Preparation, Revision 3, February 2007*

U.S. Environmental Protection Agency SW-846 Method 3520C, *Continuous Liquid-Liquid Extraction, Revision 3, December 1996*

U.S. Environmental Protection Agency SW-846 Method 3600C, *Cleanup, Revision 3, December 1996*

U.S. Environmental Protection Agency SW-846 Method 3620C, *Florisil Cleanup, Revision 3, February 2007*

U.S. Environmental Protection Agency SW-846 Method 3630B, *Silica Gel Cleanup, Revision 3, December 1996*

U.S. Environmental Protection Agency SW-846 Method 3665A, *Sulfuric Acid / Permanganate Cleanup, Revision 1, December 1996*

U.S. Environmental Protection Agency SW-846 Method 8270D, *Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS), Revision 4, February 2007.*

USEPA Region 9 Laboratory. *Business Plan*

USEPA Region 9 Laboratory. *Chemical Hygiene Plan*

USEPA Region 9 Laboratory. *Environmental Management System*

USEPA Region 9 Laboratory. *Laboratory Quality Assurance Plan*

USEPA Region 9 Laboratory. SOP 239, *Recycling Used Methylene Chloride*

USEPA Region 9 Laboratory. SOP 290, *Extraction of Soil Samples Using Pressurized Fluid.*

USEPA Region 9 Laboratory. SOP 315, *Semivolatile Organics Analysis*

USEPA Region 9 Laboratory. SOP 330, *Organochlorine Pesticides by Gas Chromatography*

USEPA Region 9 Laboratory. SOP 335, *Polychlorinated Biphenyls (PCBs) as Aroclors by GC/ECD*

USEPA Region 9 Laboratory. SOP 385, *Extractable Petroleum Hydrocarbons by GC/FID*

USEPA Region 9 Laboratory. SOP 706, *Laboratory Waste Management Procedure*

USEPA Region 9 Laboratory. SOP 840, *Notebook Documentation and Control*

USEPA Region 9 Laboratory. SOP 880, *Demonstration of Capability*

APPENDIX A.
DEVIATIONS FROM THE REFERENCE METHOD

1. This SOP uses the manufacturer's recommended water bath temperature (95°C) for the S-EVAP™ for hexane to overcome insulating effects of the apparatus. The reference method recommends a water bath temperature 15 to 20°C above the boiling point of the solvent. For hexane, the reference method bath temperature would be 84-89°C.

2. Samples for SVOC are extracted at pH <2 only; a second extraction at pH >11 as described in the reference method is not performed. It has been demonstrated that extraction at pH 2 is sufficient in recovering all target analytes.

3. Florisil cartridge phenol check solution is prepared at 0.1 µg/mL, not the 0.1 µg/L in SW-846 Method 3620C, *Florisil Cleanup*, Section 7.11. The deviation is necessary because the GC/ECD is not sufficiently sensitive to 2,4,5-trichlorophenol to detect 5% residual in the extract at the lower concentration.

APPENDIX B.
FLORISIL CARTRIDGE PERFORMANCE CHECK

1. Each lot number of Florisil cartridges must be tested before they are used for sample cleanup.

2. Add 0.5 mL of 2,4,5-trichlorophenol solution (0.1 µg/mL in acetone) and 0.25 mL of the Pesticide Mix at 0.02/0.04/0.20 µg/mL with surrogate at 0.08 µg/mL in hexane to 1.25 mL of hexane. If the analyst prepares the standards at different concentrations they must provide the volumes of standards and hexane to use for the check and the concentration of pesticides and 2,4,5-trichlorophenol in the final check solution must not be altered.

3. Place the mixture onto the top of a washed Florisil cartridge, and elute it with 9.0 mL of hexane/acetone 9:1 v/v. Reduce the final volume to 1.0 mL using nitrogen.

4. Provide the extracts and a 1.0 mL vial each of the 2,4,5-trichlorophenol solution (0.1 µg/mL in acetone) and the Pesticide Mix at 0.02/0.04/0.20 µg/mL with surrogate at 0.08 µg/mL in hexane to the analyst along with the performance check.

5. Analyze the solution by GC/ECD using at least one of the GC columns specified for sample analysis. Determine the recovery of each analyte for evaluation and reporting purposes. Calculate the percent recovery using the following equation:

$$\text{Percent Recovery} = \frac{Q_d}{Q_a} \times 100$$

Where,

Q_d = Quantity determined by analysis

Q_a = Quantity added

6. The lot of Florisil cartridges is acceptable if all pesticides and surrogates are recovered at 80 to 120 percent, the recovery of 2,4,5-trichlorophenol is less than 5 percent (compound should be retained on Florisil cartridge), and no peaks interfering with the target analytes are detected.

APPENDIX C.
LIMS FORMS

Example LIMS Analytical Standard Record

Analytical Standard Record

EPA Region 9 Laboratory

6B01012

Description:	OCP_020106SPK	Expires:	08/01/06
Standard Type:	Analyte Spike	Prepared:	02/01/06
Solvent:	Solvent Lot # Hexane	Prepared By:	Lorraine Avalos
Final Volume (mls):	10	Department:	Extractables
Vials:	1	Last Edit:	02/01/06 14:28 by LA

PEST SPIKE MADE BY LA IN Hexane, 0.2-2.0 ug/ml

Analyte	CAS Number	Concentration (ppm)
Endosulfan II	33213-65-9	0.4
4,4'-DDE	72-55-9	0.4
4,4'-DDT	50-29-3	0.4
Aldrin	309-00-2	0.2
alpha-BHC	319-84-6	0.2
alpha-Chlordane	5103-71-9	0.2
beta-BHC	319-85-7	0.2
delta-BHC	319-86-8	0.2
4,4'-DDD	72-54-8	0.4
Endosulfan I	959-98-8	0.2
Methoxychlor	72-43-5	2
Endosulfan sulfate	1031-07-8	0.4
Endrin	72-20-8	0.4
Endrin aldehyde	7421-93-4	0.4
Endrin ketone	53494-70-5	0.4
gamma-BHC (Lindane)	58-89-9	0.2
gamma-Chlordane	5103-74-2	0.2
Heptachlor	76-44-8	0.2
Heptachlor epoxide	1024-57-3	0.2
Dieldrin	60-57-1	0.4

Parent Standards used in this standard:

Standard	Description	Prepared	Prepared By	Expires	Last Edit	(mls)
4K18010	OCP:Organochlorine Pesticide Mix AB	11/18/04	** Vendor **	11/30/06	11/18/04 12:55 by LA	0.25

Example LIMS Bench Sheet

% SOLIDS BENCH SHEET

B9K0135

EPA Region 9 Laboratory

Project: -

nted: 11/23/2009 11:02:01AM

Matrix: Water **Analysis:QC** **Prepared using: Extractables - 3520B CLLE** **Surrogate used: 9K04005**

Lab Number	SampleName	Prepared	Weight 1, g	Weight 2, g			Source ID	Prepared By	Extraction Comments
B9K0135-BLK1	Blank	11/20/09 14:11						LA	
B9K0135-BS1	LCS	11/20/09 14:11						LA	MDL-7 ph5
B9K0135-BS2	LCS	11/20/09 14:11						LA	MDL-8 ph5
B9K0135-BS3	LCS	11/20/09 14:11						LA	MDL-9 ph5
B9K0135-BS4	LCS	11/20/09 14:11						LA	PA-5 ph5
B9K0135-BS5	LCS	11/20/09 14:11						LA	PA-6 ph5

MDL PCB Acid clean done : Glasswool rinsed 10/28/09. NO LOT NUMBER ON BOX. Start:15:20 Stop:09:30

Reagents

Reagent #	Description
9G28008	Sulfuric Acid
9I29022	Sodium Sulfate/49189930
9J02006	Hexane/CZ454
9K10009	Dichloromethane/LN:CZ907

Preparation Reviewed By _____ Date _____

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APPENDIX D.
PREVENTIVE MAINTENANCE REQUIREMENTS

Item	Frequency	Actions/Comments
Refrigerated recirculator	Each use	Check the water level and the system for leaks; do not proceed until all leaks are fixed. Add DI water as necessary.
	Each use	Check water temperature and flow rate while chiller is running to make sure it can maintain appropriate settings.
	Monthly	Take out and inspect the chiller filter periodically; clean filter when needed.
	Monthly	Add cap of bleach or algaecide to circulating water if water looks greenish or black.
S-EVAP™	Each use	Check the water level; it should be up to or just below drain (located inside water bath). Add water as necessary.
	Each use	Check that water flows properly and there are no plugs and that all tubing runs into drain in hood.
	Monthly	Change bath water if water looks greenish or black; this is usually not a problem if drain is not plugged.
	Annually	Check accuracy of heating unit; should be within a 1 or 2˚C of digital display temperature.
S-EVAP™ condenser	Each use	Check system for leaks; stop immediately and fix all leaks before proceeding.
	Each use	Check that water flows properly and there are no plugs and that all tubing runs into drain in hood.
N-EVAP™	Before each use	Clean needles with same solvent as extract
	Before each use	Check that all silicon tubing is in good shape and connected to all needle valves before using.
	Each use	If the gas flow rate is too slow or too great, inspect the needle valve to make sure it is opened to the appropriate amount. If flow rate problems still occur, check the nitrogen tank located in the EMFAC area; change the tank if necessary.
	Monthly	Verify that there are there enough aluminum beads in the dry media bath to come to ~1/2 inch from the top; if not, add as necessary.

APPENDIX E.
REVISION HISTORY

STANDARD OPERATING PROCEDURE: 275
Revision: 5, Effective: 12/23/2011

EXTRACTION OF WATER SAMPLES BY CONTINUOUS LIQUID-LIQUID EXTRACTION

Revision	Effective Date	Description
3	06/05/09	1. Update surrogate and matrix amounts, final volumes, etc. for consistency with SOP 290. 2. Update Florisil cartridge procedure and acid cleanup to comply with method. 3. Minor formatting revisions.
4	11/27/09	1. Added requirement to record start and stop times for CLLEs, include all reagents in the LIMS batch, and document date glass wool was prepared. 2. Added the option to directly transfer weights from the balance into a spreadsheet and then into LIMS.
5	12/23/11	1. Incorporated changes to SVOC spiking solutions. 2. Included recording time and date for start and stop of CLLEs. 3. Included balance requirements in equipment list. 4. Minor revisions to internal COC, training documentation and EMS.